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**Discovery of novel mammary developmental and cancer genes using ENU mutagenesis**

This project seeks to discover new genes involved in mammary gland carcinogenesis, by screening for reduced latency to palpable mammary tumors in response to the myc oncogene, that is produced by the loss of a second gene knocked out by random ENU mutagenesis. This project draws upon the large scale recessive ENU mutagenesis project at the John Curtin School Australian National University.

We have successfully imported, rederived and cleared from Quarantine the bi-transgeneic inducible myc model of mammary carcinogenesis devised by Prof. Lewis Chodosh. We have established and expanded the colony and we have begun production of animals to be mated with ENU mutagenised animals. We are currently approximately 2 months behind our statement of work, due mainly to a delay experienced in the importation process. On the positive side the construction of a very large new mouse facility at the Australian National University is ahead of time and is due for commissioning in April next year, which should decrease the time required for pedigree screening and expansion in 2004 and 2005.

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## *Introduction*

To date there has been no systematic search for genes controlling mammary development and carcinogenesis, and this project seeks to undertake the first such search by participation in the large scale recessive ENU mutagenesis project currently being conducted by Prof. Chris Goodnow, at the John Curtin School, Australian National University (ANU).

## *Body*

In the first annual report we proposed a modified Statement of Work that included the use of the tet/c-myc bi-transgenic model of mammary cancer, following the failure of the initial experimental plan. This modification was accepted by the reviewers, who also approved a 1 year, no cost extension of the reporting period. Thus this third annual report covers the 12 month period to Sept 2004 and refers to the second year (months 12-24) of the revised statement of work below.

### REVISED SOW 2003-2005

**Task 1.**
*Months 1 to 9.*
1. Establish Tet-myc bitransgenic mice at ANU by rederivation.
2. Establish genotyping PCRs and test.

**Task 2.** Establish and screen 200 inbred pedigrees
*Months 9 to18.*
a. Mate homozygous FVB Tet-myc bitransgenic females with 200 C57Bl6 ENU mutagenised males to establish 200 pedigrees. Keep G1 males. Simultaneously mate 20 unmutagenised males to establish 20 control pedigrees.
b. Mate G1 males to homozygous Tet-myc bitransgenic females to produce G2 offspring.
c. Mate G2 females with their fathers to produce 25 G3 females per ENU or control pedigree.
d. Induce transgene expression at 9 weeks of age with doxycycline. Monitor G3 females for mammary tumors by palpitation every 10 days. Select females with tumors from the ENU group which become palpable before tumors are seen in the control group.

**Task 3.** Establish pedigrees from selected females.
Months 12-24
a. Stop doxycycline treatment and mate tumor bearing female with father, or brother if father fails to breed. Test progeny for accelerated tumorigenesis and breed pedigree to homozygosity. Examine tumor phenotype to prioritize pedigrees for mapping.

**Task 4.** Map mutations
*Months 24-36.*
a. Mate homozygous females to male FVB to produce F2
b. Phenotype F2 and collect DNA into affected (20 animals) and unaffected (20+ animals) pools.
c. Genotype using 100 micro satellite markers polymorphic between FVB and C57Bl6 chosen throughout the genome.
d. Identify locus and regenotype using 100 locus specific polymorphic markers.

e. Continue intercross/backcross if no informative recombinations are found.
f. Identify candidate genes at a sub 1 cM locus from the mouse genome map. Exclude those not expressed in the mammary gland. Begin sequencing of remaining candidates to identify ENU-induced mutation.

**Progress in relation to the SOW.**

In the first 12 months of the amended SOW we imported the two transgenic lines from the Laboratory of A/Prof Lewis Chodosh, and had them released from quarantine. The lines have been rederived and placed behind the barrier of our SPF facility at the Garvan Institute. The colonies of each line were established and PCR based genotyping assays were established and verified. We commenced the three stage intercross of these lines to provide animals (TOM*/* MTB */ in the scheme below) suitable for breeding with ENU mutagenised animals. We estimated that we were 2 months behind our anticipated position in the SOW and anticipated transferring these animals to the new ANU facility to begin the breeding with the ENU mutagenised mice.

In the 12 months to Sept 2004 we continued to breed TOM/MTB mice ready for transfer to the ANU facility. The facility was completed on schedule mid year and is now operational, see http://www.apf.edu.au/about/ for details and a downloadable .pdf outlining the new facility. The facility is now refusing to accept our animals without rederivation into their facility, despite the demonstrated SPF status of our animals, to comply with the instructions of their newly appointed Scientific Advisory Committee. This decision of the Scientific Advisory Committee was made despite the objections of the Facility Management and was not foreseen or expected and thus not included in the SOW. As a result our project has now been delayed while rederivation procedures are put in place. The TOM/MTB mice are now scheduled to be transferred to the ANU facility in the week6-10 December 2004. This unforeseen problem has resulted in a 12 month delay in this project.

Despite these setbacks we intend to continue this work as it has the potential to discover both new mammary tumour suppressor genes and genes essential for myc action. These genes will be excellent new therapeutic targets for breast canecr. The intended experimental work is reproduced below from last year's annual report and will commence in January 2005.

Abbreviations.

TOM = tet on Myc which is the myc oncogene driven by tet on promoter.

MTB = MMTV-rtTA which is the reverse tetracycline transactivator driven by mouse mammary tumour virus LTR. Mammary specific.

*/* Homozygous transgenic animal

*/ Heterozygous transgenic animal

**Production of animals suitable for breeding to ENU mutagenised males.**

The tet inducible system is bitransgenic, requiring the intercross of the imported mice to produce animals suitable for breeding, as follows.

1.TOM*/* bred with MTB */ produces 50% TOM*/ , MTB*/

2. TOM*/ , MTB*/ bred with TOM*/* produces 25% TOM*/*, MTB*/

3. TOM*/*, MTB*/ bred with TOM*/* produces 50% TOM*/*, MTB*/ suitable for breeding with ENU mice.

**Cross with ENU mice.**

We will make two populations, a control population without ENU mutations to accurately establish the kinetics of tumour induction, and a test population to screen for ENU mutations which accelerate or prevent tumourigenesis in response to myc. We will test each for dominantly and recessively acting mutations

**Control population**

wt C57Bl6 male mated with FVB TOM*/* MTB */ female. Wt population- aim for 320 animals made from 20 pedigrees. These animals are generated first and establish the normal profile of tumour onset in response to induction of myc.

**Test population**

G1: G1 males produced by breeding ENU treated B6 males with normal B6 females. G1 Bl6 male mated with FVB TOM*/* MTB */ female (test group, begin 12 weeks after WT group begins). 16 animals per pedigree, 200 pedigrees.

**Dominant screen**

Breed and keep eight female G2 progeny that are TOM */ MTB */ =50% of females (requires average of 16 females to be bred and typed from each pedigree). All are F1 Bl6/FVB. Add doxcyclin/sucrose to drinking water at 6weeks. Observe characteristics of tumour onset in the wt population that is running 12 weeks ahead of the test group. Involves palpating mammary glands for tumours, recording onset, number, position etc. Identify animals in the test group that show accelerated tumour onset (early tumors) or resistance to tumour onset (late or no tumours). Remove Dox from water and breed affected female with WT FVB male to begin mapping.

**Recessive screen**

Keep four male and four female G2 offspring from each pedigree for breeding to G3. Generate animals as above, for both wt and ENU populations but then intercross progeny to produce G3 females homozygous for mutations. An intercross of male and females that are het for both MTB and TOM results in the following animals

| | wt | MTB*/ | MTB*/* |
|---|---|---|---|
| wt | 1/16 | 2/16 | 1/16 |
| TOM*/ | 2/16 | 4/16 | 2/16 |
| TOM*/* | 1/16 | 2/16 | 1/16 |

So 4/16 of animals are the exact genotype but 9/16 have both TOM and MTB transgenes, but the MTB*/* animals (3/16) will be poor breeders. How tumorigenesis will be effected by dosage of TOM and MTB is unknown. The system shows titratable expression with Dox, so with luck gene dosage will not dramatically effect tumour onset. It may be possible to distinguish homozygotes from hemizygotes using quantitative PCR. We will need to test this as we go in the control group. We expect that a combination of F2 FVB/Bl6 and variable gene dosage will make tumor onset more variable, but dramatic phenotypes, such as no tumors, should remain detectable. Screen and map as for the dominant screen.

## *Key research accomplishments*

• Completion of the intercross of the the two lines for production of TOM*/* MTB */ animals for breeding to ENU mutagenised males.

## *Reportable outcomes*

None to date

## *Conclusions*

So What?

If we are successful we will discover new tumor suppressor genes that are active in the mammary gland, in the case of reduced latency mutations, or genes essential for myc-induced tumourigenesis, in the case of increased latent or tumor-free pedigrees. This will have direct relevance to breast cancer, providing potential markers of prognosis and new targets for therapy.

## *Appendices*

None